CONTROL OF BACTERIA IN VASE WATER AND QUALITY OF CUT FLOWERS AS INFLUENCED BY SODIUM DICHLOROISOCYANURATE, 1,3-DICHLORO-5,5-DIMETHYLHYDANTOIN, AND SUCROSE

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CONTENTS

TABLES

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By Francis J. Marousky[1]

ABSTRACT

Of various disinfectants evaluated for antibacterial activity in cut-flower water, the slow-release chlorine compounds sodium dichloroisocyanurate (DICA) and 1,3-dichloro-5,5-dimethyhydantoin (DDMH) consistently maintained low counts of bacteria, remained in solution, and maintained clear solutions. A 5-minute exposure to 50 mg DICA or DDMH/l was sufficient to kill cells of 10 of the 13 bacterial species tested. Greatest improvement in cut-flower quality (weight retention, turgidity) and longevity was obtained when DICA or DDMH was combined with sucrose. At low concentrations (50 mg/l), DICA and DDMH were not injurious to cut flowers, but at higher levels (above 100 mg/l), they caused foliar chlorosis of chyranthemums, snapdragons, and roses. The injury was not serious, considering the benefits of bacterial control and improvement in flower quality when DICA or DDMH was combined with sucrose. **KEY WORDS: cut flowers, cut-flower bacteria, cut-flower longevity, cut-flower quality, sodium dichloroisocyanurate, 1,3-dichloro-5,5-dimethylhydantoin, sucrose, vase water.**

SECTION 1.—INTRODUCTION

The presence of bacteria in cut-flower vase water has been amply documented (*1, 8, 9, 19*).[2] Aarts (*1*), who demonstrated the effects of bacteria on the wilting and longevity of cut flowers, concluded that bacteria act both directly on cut flowers by physically blocking the stems and indirectly by producing substances absorbed by the flowers. Ford et al. (*9*) showed that most of the micro-organisms associated with cut flowers are soil- and water-borne bacteria. Dansereau and Vines (*8*) showed that bacteria infiltrate and move within the stems of cut snapdragons. Those results confirmed Aarts' premise, implying that a large population of bacteria may physically plug the flower stem. Taplin and Mertz (*19*) reported the presence of excessively high microbial populations in vase water (10^{13}/ml). More importantly, they identified bacterial species (pathogens) that could cause diseases in humans. Taplin and Mertz concluded, "In view of the high percentage of hospital infections caused by gram-negative bacteria and the frequency of reports of infections caused by those which are not of human origin, we believe that flowers should not be introduced to high-risk areas."

The physiology of cut flowers usually is studied in the laboratory, where water is changed

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[2] Italic numbers in parentheses refer to items in "Literature Cited" at the end of this paper.

frequently and bactericides, such as silver or quinoline salts, are used. In experimental work the microbial population has been kept to a minimum and micro-organisms have not been a factor in the quality of cut flowers. The usual recommendations given to the florist have been based on such laboratory data. In commercial floristry, however, flower handling is different; many flower stems are arranged in a relatively small container with a small volume of water. In these circumstances contamination is high. In the vase water of floral arrangements, bacterial population can quickly reach as high as 10^7 cells/ml (*19*). Preservatives have been recommended for prolonging flower life and reducing the microbial population in vase water. Although florists use floral preservatives, many still complain about "odoriferous," "cloudy," or "stale" water. The most common preservatives used currently in commercial trade and research have had as a principal ingredient quinoline salts, or their derivatives, and sucrose (*5, 7, 13, 16, 17*).

The quinoline compounds can be considered inhibitors of bacteria rather than bactericides (*8, 12*). Larson and Cromarty (*12*) isolated bacteria from flower solutions containing 300 parts per million 8-hydroxyquinoline citrate (8-HQC). Dansereau and Vines (*8*) showed that *Pseudomonas marginata* and *Flavobacterium lutescens* were not inhibited when subjected to an agar medium containing 600 parts per million 8-HQC. Marousky (1975, unpublished data) demonstrated that *Erwinia* species grew well in a solution containing 8-HQC plus sucrose but poorly in nutrient broth plus 8-HQC. Albert et al. (*2, 3*) worked extensively on the mechanism of action of 8-hydroxyquinoline (oxine). They showed that the chelating complex of 8-hydoxyquinoline with divalent metals was the basis for its antibacterial activity (*3*) and that 8-hydroxyquinoline had no antibacterial activity in the absence of iron or copper (*2*).

Several attempts have been made to find chemicals that will reduce or kill the bacteria in cut-flower water (*1, 8, 12, 21*).

Wiggins and Payne (*21*) demonstrated that antibiotics did not lengthen flower life, concluding that the commercial flower preservative they used was more effective in increasing flower life than was any of the antibiotics tested. They did not make any bacterial counts from the water or any of the solutions tested.

Dansereau and Vines (*8*) demonstrated that the bacterial species they isolated from cut-flower water were sensitive to sulfacetamide, demethylchortetracycline, and nalidixic acid. They concluded that specific antibiotics appear highly effective in vitro, but they did not test any antibiotics in vivo (that is, on cut flowers).

Smellie and Brincklow (*18*) reported that chlorhexidine diacetate prevents the formation of maloderous cut-flower water and that the chemical was not toxic to cut flowers.

Kofranek et al. (*10*) demonstrated that an organic stabilized slow-release chlorine compound with sucrose was effective in maintaining cut-flower quality and longevity. They concluded that the chlorine compound tested was an effective antimicrobial agent, but they did not make any bacterial counts. "Active" chlorine compounds have strong disinfecting properties and have been used in commercial industrial products for many years (*14*).

Silver salts, long used as bactericides in medicine and industry (*14*), have also been used on cut flowers (*1, 11*). Aarts (*1*) used silver salts to attain satisfactory control of bacteria in cut-flower water. Kofranek and Paul (*11*) recently used silver salts with sucrose to enhance flower life. The effectiveness of silver salts as bactericides in the laboratory is indisputable, but there are also necessary practical considerations. Silver is usually used as the acetate or nitrate salt, and under these circumstances the salts photooxidize and precipitate a black suspension that coats the flower stems. Unless the salts are used with chloride-free water, silver ions are precipitated as silver chloride. Most of the water supplies in the United States are chlorinated. Although silver salts are effective in the laboratory as bactericides, the secondary effects of their use might raise questions whether they would be acceptable in commercial practice. The objectives of this study were to determine the level of bacteria commonly encountered in cut-flower water and to survey, screen, and evaluate promising bactericides for elimination and control of bacteria normally found in cut-flower water.

SECTION 2.—SCREENING BACTERICIDES FOR CUT-FLOWER WATER

METHODS AND MATERIALS

One to six disinfectants or chemicals representing various groups of bactericides commonly

used in medicine and industry were selected for evaluation (*14*). Chemical agents were selected on the following basis: (a) each chemical had known bactericidal properties and was readily available, (b) each chemical was water soluble and remained in solution, (c) each chemical acted rapidly as a bactericide. Although the chemicals were tested singly, it was hoped that chemicals in one group or category of bactericide-disinfectant would reduce cut-flower bacteria more effectively than chemicals in other groups of bactericides. If so, other chemicals in the appropriate category could also be evaluated and tested for phytotoxicity.

In preliminary tests, the chemicals were not evaluated on flowers but were tested in an aqueous system on a mixed culture of cut-flower bacteria. Various concentrations of chemicals were placed in flasks containing 1 liter of unsterilized de-ionized water. A mixed bacterial inoculum was collected from "old" cut-flower water ($1–3\times10^6$ bacteria/ml). One milliliter of inoculum was introduced into each solution, providing an approximate microbial count of 1,000 to 3,000 cells/ml of final solution. These solutions were incubated for 1 hour at 23° C. A 1-ml sample was withdrawn with a sterile pipette, and the liquid placed on sterile petri plates. Twenty milliliters of warm liquid tryptic soy agar was added to each plate. The plates were swirled, cooled, inverted, and incubated at 37° C for 48 hours. A control flask without any chemicals was inoculated and handled with each test. Colonies on each plate were counted and referred to as bacteria per milliliter. With this procedure, many chemicals could be screened for possible bactericidal properties against mixed cut-flower bacteria. A chemical was arbitrarily considered effective if there was at least a 99-percent reduction in the number of bacteria per milliliter, compared to the control sample. No antibiotics were tested, because they do not necessarily provide adequate control of cut-flower bacteria (*8*). Also, bacteria found in cut-flower water develop resistance to antibiotics (*19*). Chemicals considered effective were then tested in vivo.

Various concentrations of the test chemical were placed in glass quart jars containing 500 ml of solution and inoculated with 2 ml of water from containers holding 7- to 8-day-old cut flowers (3×10^6 bacteria/ml). Three chrysanthemum stems (45 to 50 cm long) were placed in each container. After 4 to 6 days, a 0.1-ml aliquot of each test solution or water was collected. The aliquot was handled aseptically, diluted, and plated as previously described. Before the water samples were collected, the flowers were removed from each jar, and the solutions were stirred. A small sample size was used because (a) it was representative of the bacterial population of the container, (b) it allowed for rapid and convenient dilution, and (c) it provided a negligible amount of chemical on the final dilution plates.

Leaves were rated for chlorosis and necrosis according to the following scale: 1=no chlorosis, green leaves; 2=incipient chlorosis, slight yellow mottling of leaves; 3=moderate chlorosis, large areas between the veins were yellow; 4=moderate chlorosis with necrotic leaf margins; 5=necrotic leaves. In a few instances bacterial counts were not made when leaf chlorosis was severe. A few chemical agents were tested on flowers without being screened in vitro. In these cases, bacterial counts were made as outlined above. The laboratory was held at 23° C with 1.5 klx of light supplied by cool-white fluorescent tubes for 12 hours each day. Relative humidity ranged from 40 to 70 percent.

RESULTS

Water from control flasks had numerous bacterial colonies which covered the surface of the medium and could not be counted accurately (table 1); in these instances the bacteria per milliliter are referred to as numerous. Many of the chemicals were effective in eliminating bacteria even though the water was initially not sterilized. The most effective groups of chemicals were the silver and chlorine compounds.

Chlorine dioxide and sodium hypochlorite reduced bacteria to low levels but did not completely eliminate them. Both chlorine dioxide and sodium hypochlorite were phytotoxic to chrysanthemums. Leaves on stems held in chlorine dioxide (10 mg/l) for 7 days were severely injured, and leaves on stems held in sodium hypochlorite (50 mg/l) for 7 days were only moderately injured. The severity of injury or chlorosis increased as the concentration of sodium hypochlorite increased.

The slow-release chlorine compounds, sodium dichloroisocyanurate (DICA) and 1,3-dichloro-5,5-dimethylhydantoin (DDMH), and *p*-sulfondichloroamidobenzoic acid (halazone) were highly effective, nearly reducing bacterial counts to zero. Both DICA and DDMH at 50

TABLE 1.—*Influence of various chemicals on bacterial populations in cut-flower water and on chlorosis of chrysanthemum leaves*

Chemical	Highest concentration (mg/l)	Screening test (bacteria/ml)	Chrysanthemum test remarks
Water (control)		(1)	10^6 bacteria/ml after 4 days; no chlorosis after 14 days, but leaves severely wilted.
Metals:			
Ag^+ as $AgNO_3$	20	0	10^3 bacteria/ml after 6 days; no phytotoxicity, stem ends coated with a black precipitate.
Ag^+ as mild Ag-protein	20	0	Good control of bacterial; no precipitation; no phytotoxicity at 10 mg/l; solution dark brown to deep yellow depending on water quality.
Cu^{++} as $CuSO_4$		...	10 to 80 mg Cu^+ as $CuSO_4$ did not provide any bacterial control; injured stems severely and caused leaf chlorosis.
Phenols:			
O-phenylphenol	200	(1)	Not tested on flowers.
p-chloro-*m*-cresol	200	180	Do.
p-tert-amylphenol	200	96	Not tested on flowers, not completely soluble.
8-hydroxyquinoline citrate.	200	(1)	10^6 bacteria/ml after 4 days.
Chlorine compounds:			
Dichloroisocyanuric acid	50	0	Not tested on flowers.
Sodium dichloroisocyanurate.	50	0	No chlorosis after 14 days; only one colony at a dilution of 10^{-2}.
Trichloroisocyanuric acid	50	0	Not tested on flowers.
1,3-dichloro-5,5-dimethylhydantoin.	50	0	No chlorosis after 14 days; two colonies at 10^{-2} dilution.
Chlorine dioxide	20	0	9.5×10^4 bacteria/ml after 4 days at 10 mg/l; moderate chlorosis at 7 days and severe chlorosis at 10 days.
Sodium hypochlorite	50	0	1.5×10^3 bacteria/ml after 4 days at 50 mg/l; higher levels produced earlier and greater chlorosis.
p-sulfondichloroamidobenzoic acid.	10	2	Material dissolved in sodium carbonate solution; must remain in alkaline medium to remain soluble.
Iodine compounds:			
I^+ as KI		...	10^6 bacteria/ml at 10 to 40 mg I^+/l, no bacterial control or phytotoxicity.
I^+ as KI + 8-hydroxyquinoline citrate.		...	20 mg of I^+ with 8-HQC, bacteria population reduced to 10^3 ml, with no phytotoxicity.
Miscellaneous:			
Hydrogen peroxide	320	36	Can control bacteria, but at high concentrations caused a foliar chlorosis.
Dehydro acetic acid	200	(1)	Not tested on flowers.
Chlorhexidine diacetate.	200	3	10^6 bacteria/ml after 6 days; phytotoxic to chrysanthemum leaves.

[1] Numerous: colony covered the surface of the medium and could not be counted accurately.

mg/l maintained a low count of bacteria (1 to 2 colonies at a 10^{-2} dilution) in water. There was no chlorotic foliage on chrysanthemums after 14 days. Halazone was soluble in Na_2CO_3 solution (*14*) and was effective in maintaining a low bacterial count in water, but the results were not as consistent as with DICA and DDMH. Mold frequently grew on flower stems in halazone but not on flower stems held in solutions of DICA and DDMH.

Silver salts were highly bactericidal. Silver, which was tested as $AgNO_3$, precipitated and turned black, coating the ends of flower stems. Precipitation occurred in de-ionized water and

began overnight. Silver as Ag-protein provided adequate control of bacteria equal to $AgNO_3$. The Ag-protein solution was a dark brown to deep yellow color at 10 to 20 g silver/l, did not precipitate, and was not phytotoxic to chrysanthemums. Additional Ag-protein was also tested on snapdragons and maintained good control of bacteria. Ag-protein at 20 mg Ag/l was injurious to snapdragon leaves (necrosis of the leaf margins).

Copper (up to 80 mg Cu^{++}/l as $CuSO_4$) did not control bacteria and was phytotoxic to chrysanthmeums. Copper sulfate turned chrysanthemum stems brown and caused severe foliar chlorosis.

Iodine (up to 40 mg/l as KI) did not control bacteria and was not phytotoxic to chrysanthemums. Mixed with 200 mg 8-HQC/l, it was effective in reducing the bacterial population to 10^3 cells/ml.

Hydrogen peroxide (320 mg/l) was effective in controlling bacteria, but at this level it injured chrysanthemum foliage. At levels less than 320 mg/l, hydrogen peroxide reduced but did not eliminate bacteria and was not as injurious to foliage as at higher levels.

Chlorhexidine diacetate controlled bacteria in initial screening tests, but it did not adequately control the bacterial population in water holding chrysanthemum stems. At 200 mg/l it was phytotoxic to chrysanthemums, and bacteria in vase water reached 4.2×10^6 cells/ml. Phytotoxicity was observed first as a slight chlorosis of the leaves, and eventually the entire leaf became chlorotic with necrotic leaf margins.

SECTION 3.—SPECIFIC TESTS ON CUT FLOWERS WITH CHLORINATING CHEMICALS

METHODS AND MATERIALS

Cut flowers were placed in de-ionized water or solutions containing 50 mg DICA or DDMH/l. Since sucrose increases flower quality and longevity (*1, 11, 13, 16, 17*), each chemical was also tested with 20 g of sucrose. Some flowers were held in 200 mg of 8-HQC+20 g sucrose/l. After the flowers had been placed in the containers, the water and solutions were inoculated with mixed bacterial cultures derived from cut-flower vase water. Size of container, amount of water and solution, and inoculum varied with the flower species used in each test. There were four flower stems per container, except with gypsophila (one bunch) and roses (three stems). All flowers were kept at 23° C throughout the tests; relative humidity was not controlled and varied from 45 to 70 percent throughout the tests; and light was maintained at 1.5 klx by cool-white fluorescent tubes for 12 hours daily. Bacteria were estimated by taking a 0.1-ml aliquot of water or solution and diluting it in sterile blanks. One milliliter of diluted sample was added to sterile petri plates, and then 20 ml of liquid tryptic soy agar was added. The plates were swirled carefully, cooled, and inverted and then were incubated for 48 hours at 37° C, after which bacterial colonies were counted on each plate. (Data are expressed as bacteria or cells per milliliter.) The sample period for solution and water collection varied with the species of flower. Flowers were weighed initially and periodically during each test, and the results are expressed as the percentage of change from initial fresh weight.

Roses.—Three 'Forever Yours' roses (35 to 38 cm long) were placed in pint glass jars containing 250 ml of water or solution. Each container was inoculated with 1 ml of mixed inoculum containing 3.5×10^6 cells/ml. Water and solution samples were collected after 3 days and bacteria per milliliter estimated.

Asters.—'Purple King' asters were trimmed to 45 cm and placed in quart glass jars containing 500 ml of water or solution. The water and solutions were not inoculated, but the flowers had been held in a common water supply in a commercial packinghouse for 3 to 5 hours. After 7 days, flower quality was evaluated on the following scale: 1=excellent quality, no signs of deterioration; 2=good quality, slight petal browning or incipient petal wilting; 3=moderate quality, petal browning, wilting, flower heads beginning to droop; 4=poor quality, flower heads drooping, petals brown. Bacteria were counted in the water and solutions after 3 days.

Carnations.—Freshly cut 'Elegance' carnations were placed in quart glass jars containing 250 ml of water or solution. Each container was inoculated with 1 ml of mixed inoculum containing 3.5×10^6 cells/ml. Bacteria were counted in the water and solutions after 5 days.

Gypsophila.—Freshly harvested *Gypsophila paniculata* (babysbreath) was grouped in bunches (about 400 to 500 g/bunch). Each bunch was placed in a quart jar filled with water or solution. Water and solution samples were

collected after 4 days. Floret quality was evaluated after 4 and 5 days as for asters. Bacteria were counted in the water and solutions after 4 days.

Gladiolus.—Spikes of 'Peter Pears' gladiolus were harvested and held overnight at 8° C. The following morning the spikes were trimmed to 60 cm in length and placed in quart glass jars containing 500 ml of water or solution. Containers were inoculated with 2 ml of mixed inoculum containing 3.5×10^6 cells/ml. Bacteria in water and solutions were counted after 6 days. Sucrose was added at 40 g/l (*15*) rather than at 20 as in the previous tests.

RESULTS

Typically, cut-flower water reached a bacterial population of 10^6 cells/ml in 3 or 4 days, and water with more than 10^6 bacteria/ml was usually cloudy or turbid. Cut-flower water containing 8-HQC+sucrose had high bacterial counts. The chlorinating chemicals eliminated or greatly reduced bacterial populations in water. Cut-flower quality, turgidity, and weight were maintained only when the chlorinating chemicals were combined with sucrose.

Roses.—Water holding roses for 3 days contained a large bacterial population (table 2). The cholorinated solutions with or without sucrose were free or essentially free of bacteria, while the 8-HQC+sucrose solution had a high population of bacteria (6.9×10^5 cells/ml). In water, some roses wilted by the third or fourth day, and after 5 days all had wilted; flowers did not open and inner petals turned blue. The roses held in the chlorinating chemicals with sucrose maintained turgidity, weight, and red petal color, but without sucrose they developed blue petals. No symptoms of phytotoxicity were noted for DICA and DDMH at the levels tested.

Asters.—In water, asters deteriorated rapidly; stem ends became slimy and stem tissue sloughed off. The bacterial population reached 3.1×10^6 cells/ml in 3 days (table 3) and was also large in the 8-HQC+sucrose solution. The

TABLE 2.—*Influence of DICA and DDMH with and without sucrose, and 8-HQC plus sucrose on bacterial populations in vase water and on weight change of* 'Forever Yours' *roses*

Chemical concentration/l	Bacteria/ml after 3 days[1]	Percent weight change after—		
		2 days	4 days	6 days
Water (control)	1.5×10^6	−3	−7	−20
50 mg DICA	0	+5	+2	−12
50 mg DICA+20 g sucrose	0	+5	+10	+12
50 mg DDMH	15	+7	+1	−10
50 mg DDMH+20 g sucrose	50	+8	+9	+6
200 mg 8-HQC+20 g sucrose	6.9×10^5	+3	+3	−16

[1] Lowest dilution was 10^{-1}.

TABLE 3.—*Influence of DICA, DDMH, and 8-HQC with and without sucrose on bacterial populations in vase water, weight change, and quality of* 'Purple King' *asters*

Chemical concentration/l	Bacteria/ml after 3 days[1]	Percent weight change after—		Quality after 7 days[2]
		7 days	10 days	
Water (control)	3.1×10^6	−16	−43	3.8
50 mg DICA	0	−9	−48	3.7
50 mg DICA+20 g sucrose	0	+25	+24	1.0
50 mg DDMH	3	−7	−34	3.8
50 mg DDMH+20 g sucrose	0	+32	+35	1.0
200 mg 8-HQC	1.6×10^6	−11	−36	3.2
200 mg 8-HQC+20 g sucrose	4.4×10^6	−4	−17	2.4

[1] Lowest dilution was 10^{-1}.

[2] 1=excellent; 4=wilted, deteriorated flowers.

chlorinating chemicals greatly reduced or eliminated bacteria. 8-HQC+sucrose increased flower size and quality but not to the same degree as DICA and DDMH with sucrose. After 7 days, the asters in the chlorinated solutions with sucrose were turgid; the inner petals had expanded; and the overall sizes of the flowers had increased. Without sucrose there was no apparent stem breakdown or injury to any of the flowers, but all stems held in sucrose-based solutions had chlorotic leaves.

Carnations.—Water and 8-HQC+sucrose holding carnations had large bacterial populations. The chlorinating chemicals reduced the bacterial population but did not eliminate all bacteria (table 4). Water containing DICA or DDMH with sucrose had twice the bacterial population of water containing DICA or DDMH without sucrose. Carnations held in DICA or DDMH with sucrose were of superior quality, had more open florets per stem, and lasted longer than flowers held in water or DICA or DDMH without sucrose. Florets on stems held in chlorinating chemicals with sucrose were superior in quality and longevity than florets on stems held in 8-HQC+sucrose.

Gypsophila.—DICA or DDMH with or without sucrose or 8-HQC+sucrose were not effective in eliminating or reducing bacteria (table 5). The bacterial population in water reached 10^7 cells/ml in 4 days. Stems held in DDMH and DICA with sucrose or 8-HQC+sucrose had more open florets than stems held in plain water or DICA or DDMH without sucrose. After 4 days stems in all solutions began to break down, and water and all solutions developed a putrid odor. Florets deteriorated rapidly after 4 days, losing turgidity and turning brown, and eventually the entire bunch collapsed.

Gladiolus.—Water holding spikes was clear and contained a relatively low bacterial population (table 6), and water containing 8-HQC+sucrose had a higher bacterial population. Water containing DICA or DDMH without sucrose had relatively few bacteria, and water containing DICA or DDMH plus sucrose had slightly more bacteria. Spikes held in chlorinating chemicals with sucrose and 8-HQC+sucrose were superior in quality and weight than spikes held in water or chlorinating chemicals without sucrose. Spikes held in 8-HQC+sucrose or DICA or DDMH+sucrose had more and larger florets per spike than those held in water. Spikes held in water had the poorest quality and were the first spikes to wilt.

SECTION 4.—EVALUATION OF CHEMICALS AGAINST SPECIFIC BACTERIA

METHODS AND MATERIALS

An in vitro procedure was established to test the bactericidal properties of DICA and DDMH on bacteria that have been found in cut-flower vase water. These include common soil- and water-borne bacteria (*9*) and bacteria pathogenic to humans (*19, 20*). Also included was a strain of *Erwinia* species isolated from chrysanthemum vase water (Marousky, 1975, unpublished data). The procedure was a modification of that used by the Association of Official Agricultural Chemists (*4*). One milliliter of 0.05 g/100 ml sterile solution of each chemical was added to 9.0 ml of sterile water in stoppered test tubes, providing a final concentration of 50 mg/l.

TABLE 4.—*Influence of DICA and DDMH with and without sucrose, and 8-HQC plus sucrose on bacterial populations in vase water, weight change, and quality of* 'Elegance' *carnations*

Chemical concentration/l	Bacteria/ml after 5 days	Percent weight change after—		Viable florets per stem
		2 days	5 days	at 9 days
Water (control)	4.5×10^6	+7	−8	(1)
50 mg DICA	5.5×10^2	+10	+5	0.8
50 mg DICA+20 g sucrose	2.2×10^4	+13	+12	2.5
50 mg DDMH	4.0×10^2	+10	+5	.2
50 mg DDMH+20 g sucrose	1.2×10^4	+11	+14	3.0
200 mg 8-HQC+20 g sucrose	1.1×10^6	+9	−12	.5

[1] Stems discarded after 7 days because of deterioration.

TABLE 5.—*Influence of DICA and DDMH with and without sucrose, and 8-HQC plus sucrose on bacterial populations in vase water and quality of gypsophila*

Chemical concentration/l	Bacteria/ml after 4 days	Quality after[1]— 4 days	5 days
Water (control)	2×10^7	3.0	4.0
50 mg DICA	3×10^6	2.5	3.5
50 mg DICA + 20 g sucrose	7×10^6	1.0	2.5
50 mg DDMH	6×10^6	2.5	3.5
50 mg DDMH + 20 g sucrose	9×10^6	1.0	1.0
200 mg 8-HQC + 20 g sucrose	1×10^6	1.0	2.0

[1] 1 = excellent; 4 = wilted, deteriorated flowers.

TABLE 6.—*Influence of DICA and DDMH with and without sucrose, and 8-HQC plus sucrose on bacterial populations in vase water and on fresh weight changes of* 'Peter Pears' *gladiolus*

Chemical concentration/l	Bacteria/ml after 6 days[1]	Percent weight change after— 6 days	7 days
Water (control)	3.0×10^4	+1	−13
50 mg DICA	15	+28	+16
50 mg DICA + 40 g sucrose	450	+33	+23
50 mg DDMH	15	+27	+13
50 mg DDMH + 40 g sucrose	150	+34	+24
600 mg 8-HQC + 40 g sucrose	4.1×10^6	+37	+29

[1] Lowest dilution was 10^{-1}; colonies did not form on all plates.

These test tubes were inoculated with 0.01 ml of a 2- to 3-day-old nutrient broth culture containing specific micro-organisms listed in table 7. Control tubes consisted of 10 ml of sterile water and 10 ml of 8-HQC equivalent to 200 mg/l. The 8-HQC solution was initially 0.20 g/100 ml, and it was diluted in a similar manner as the chlorinating chemicals. All chemical solutions were filtered through micropore filters. All test tubes were inoculated and after 1, 5, and 30 minutes, a sterile loop was inserted into each tube. The loop was transferred aseptically to test tubes containing 20 ml of sterile nutrient broth. Nutrient broth was incubated at temperatures optimum for the various micro-organisms (*6*). Test tubes were observed after 48 and 96 hours. Any evidence of turbidity or change in nutrient broth was indicative of growth and received a plus (+) score. This procedure was repeated several times with three replications each time. If a single "plus" value was recorded for any replication, then the entire treatment was recorded as "plus."

RESULTS

DICA and DDMH were effective in controlling various species of bacteria typically found in cut-flower water (table 7). A 1-minute exposure to 50 mg DICA or DDMH/l was sufficient to kill most bacteria. Only cells of 3 of the 13 bacterial species tested were not killed in a 5-minute period. None of the bacteria tested survived a 30-minute exposure to DICA or DDMH. All of the bacteria exposed to 8-HQC or held in water for 30 minutes survived and grew well on nutrient broth.

SECTION 5.—TOXICITY OF CHLORINATING CHEMICALS

METHODS AND MATERIALS

The two chlorinating chemicals, DICA and DDMH were tested at higher concentrations to determine phytotoxicity. DICA and DDMH were tested at 100, 200, or 300 mg/l, alone or in combination with 20 g of sucrose. Exceptions to these rates are noted below. Controls consisted of water or 20 g sucrose/l. De-ionized water was used for controls and all solutions. Flower handling and record collection was similar to that described in section 3. A more stringent microbiological assay test was used. The flowers were removed from the container, and a sterile loop was introduced into the water or solution. The loop was transferred aseptically into sterile test tubes containing 20 ml of nutrient broth, and these tubes were incubated at 24° C for 96 hours. Tubes were noted after 48 and 96 hours. Microbial growth was recorded subjectively as: (a) + = possibility of growth; (b) ++ = clear indication of growth (turbidity or cloudiness of broth); (c) +++ = heavy growth; (d) − = no growth, clear broth after the 96-hour incubation period. A plus score for any vase (replication) of a given treatment was scored as plus for the entire treatment even though the other vases (replications) from that treatment may have had tubes with no evidence of bacterial growth.

Roses.—Stems of 'Forever Yours' roses were trimmed to 30 to 35 cm in length and placed in

TABLE 7.—*Growth of various bacterial species after exposures of 1, 5, and 30 minutes to DICA, DDMH, and 8-HQC*

Bacterium	Incubation temperature (° C)	Water (control) exposure time (min)			DICA[1] exposure time (min)			DDMH[1] exposure time (min)			8-HQC[2] exposure time (min)		
		1	5	30	1	5	30	1	5	30	1	5	30
Escherichea coli	37	+	+	+	−	−	−	−	−	−	+	+	+
Pseudomonas fragi	30	+	+	+	−	−	−	−	−	−	+	+	+
Bacillus cereus	30	+	+	+	+	+	−	+	+	−	+	+	+
Bacillus subtilis	37	+	+	+	−	−	−	+	+	−	+	+	+
Micrococcus luteus	23	+	+	+	−	−	−	−	−	−	+	+	+
Aerobacter aerogenes	30	+	+	+	−	−	−	+	−	−	+	+	+
Pseudomonas aeruginosa No. 62[3]	37	+	+	+	−	−	−	+	+	−	+	+	+
Aeromonas hydrophila	37	+	+	+	−	−	−	−	−	−	+	+	+
Micrococcus roseus	23	+	+	+	−	−	−	−	−	−	+	+	−
Klebsiella pneumoniae	37	+	+	+	−	−	−	−	−	−	+	+	+
Pseudomonas cepacia No. 96[4]	37	+	+	+	+	−	−	−	−	−	+	+	+
Pseudomonas cepacia No. 143[4]	37	+	+	+	−	−	−	−	−	−	+	+	+
Erwinia species	37	+	+	+	−	−	−	−	−	−	+	+	+

[1] 50 mg/l.
[2] 200 mg/l.
[3] Obtained courtesy of M. N. Schroth, University of California, Berkeley, Calif. 94720.
[4] Obtained courtesy of D. Taplin, University of Miami, Miami, Fla. 33152.

250 ml of water or solution in pint glass jars. Four flowers were placed in each jar and the water or solutions inoculated with 1 ml of old vase water (about 10^6 cells/ml).

Carnations.—Pink standard carnations were purchased from a local wholesale florist. Cultivar and cultural information were not available, but flowers were airshipped from Colombia, South America. After the flowers were kept overnight in water in common storage at 8°–10° C, the stems were trimmed to 60 cm in length and placed in quart glass jars containing 500 ml of water or solutions. Each vase was inoculated with 2 ml of old vase water (10^6 cells/ml).

Snapdragons.—Freshly cut 'Oklahoma' spikes were harvested when one-third of the florets were open. Spikes were trimmed to 45 cm in length and placed in groups of three in quart glass jars containing 250 ml of water or solutions. Florets were considered open when the two upper petals were erect and parallel to the longitudinal axis of each floret, and wilted when petals became discolored or when petal edges began to curl or loose turgidity.

Gladiolus.—'White Friendship' gladiolus spikes were graded and kept dry overnight at 8°–10° C and then placed in plastic containers (20 cm wide by 35 cm high) holding 1 liter of water or solution. There were three spikes per container and two containers per treatment. No water or solution samples were taken for bacterial assay, and sucrose was used at a 40 g/l level (*15*).

Chrysanthemums.—Stems of 'Blue Marble' chrysanthemums were trimmed to 45 to 50 cm in length and placed in quart glass jars containing 250 ml of water or solution. Chemical solutions consisted of 150 mg of DICA or 150 mg of DDMH factorially arranged with 0 or 20 g sucrose/l of water. Water and sucrose (20 g/l) were used as controls. Foliar chlorosis was recorded and bacteria/per milliter determined as outlined in section 3.

RESULTS

Roses kept in 100, 200, or 300 mg of DICA or DDMH with 20 g sucrose/l retained weight and quality longer than roses held in chemicals at similar concentrations without sucrose (table 8). Flowers responded to DICA and DDMH as described in section 3, those held in 200 mg or 300 mg DICA or DDMH/l being injured. Initially, the margin of the leaflet was injured or desiccated, and eventually the entire leaflet became necrotic. Outer petals also became desiccated, and gradually the entire petal turned brown. There was no apparent injury to the inner petals. However, little or no injury occurred at 300 mg/l

when the chemicals were combined with sucrose. Flowers held in water lasted 5 or 6 days, and those in chlorinating chemicals with sucrose lasted 8 or 9 days and did not turn blue. All levels of DICA and DDMH without sucrose controlled bacteria. With sucrose, only 300 mg DICA/l and 100 to 300 mg DDMH/l controlled bacteria.

Carnations.—No injury or phytotoxicity was observed in flowers which had been in DICA or DDMH. Flowers held in 100 to 300 mg DICA or DDMH with sucrose sustained greater turgidity, weight, and quality than flowers held in water or in DICA or DDMH without sucrose (table 9), but flower life was not appreciably increased by the prevention of bacterial growth. Sucrose alone increased flower life but not as much as

TABLE 8.—*Influence of sucrose, and three rates of DICA and DDMH with and without sucrose on bacterial growth, weight change, and petal color of* 'Forever Yours' *roses*

Chemical concentration/l	Bacterial growth[1]	Percent weight change after—			Petal color
		2 days	5 days	7 days	
Water (control)	+ + +	+6	−5	−20	Blue
20 g sucrose	+ + +	+3	+5	−2	Red
100 mg DICA	−−	+10	+8	−6	Blue
200 mg DICA	−	+9	+5	−12	Blue
300 mg DICA	−	+9	+3	−13	Blue
100 mg DICA+20 g sucrose	+ +	+7	+15	+12	Red
200 mg DICA+20 g sucrose	+	+9	+22	+15	Red
300 mg DICA+20 g sucrose	−	+11	+23	+26	Red
100 mg DDMH	−	+11	+6	−9	Blue
200 mg DDMH	−	+10	+2	−12	Blue
300 mg DDMH	−	+9	−2	−19	Blue
100 mg DDMH+20 g sucrose	−	+9	+22	+15	Red
200 mg DDMH+20 g sucrose	−	+10	+18	+8	Red
300 mg DDMH+20 g sucrose	−	+11	+22	+13	Red

[1] + = possibility of growth; + + = clear indication of growth; + + + = heavy growth; − = no growth.

TABLE 9.—*Influence of sucrose, and three rates of DICA and DDMH with and without sucrose on bacterial growth, weight change, and number of living carnation flowers*

Chemical concentration/l	Bacterial growth[1]	Percent weight change after—		Number of living flowers after[2]—		
		2 days	5 days	7 days	8 days	11 days
Water (control)	+ + +	+22	+19	3	0	0
20 g sucrose	+ + +	+23	+24	7	6	1
100 mg DICA	−	+23	+27	5	1	0
200 mg DICA	−	+22	+25	5	1	0
300 mg DICA	−	+24	+25	4	1	0
100 mg DICA+20 g sucrose	+ +	+23	+29	7	7	4
200 mg DICA+20 g sucrose	−	+24	+33	8	7	7
300 mg DICA+20 g sucrose	−	+25	+33	8	8	8
100 mg DDMH	−	+22	+25	5	1	0
200 mg DDMH	−	+21	+27	7	2	0
300 mg DDMH	−	+21	+23	4	1	0
100 mg DDMH+20 g sucrose	−	+25	+32	8	7	7
200 mg DDMH+20 g sucrose	−	+25	+33	8	8	6
300 mg DDMH+20 g sucrose	−	+23	+35	8	7	7

[1] + = possibility of growth; + + = clear indication of growth; + + + = heavy growth; − = no growth.

[2] Maximum number of living flowers = 8.

sucrose combined with DICA or DDMH. All concentrations of chlorinating chemicals with or without sucrose controlled bacteria, except 100 mg DICA+20 g of sucrose/l.

Snapdragons.—Spikes held in DICA or DDMH with sucrose had greater quality and more viable florets than those held in water, sucrose alone, or DICA or DDMH without sucrose (table 10). Flowers held in water or sucrose wilted rapidly and were discarded after 6 days. Stems were bleached by DICA and DDMH, eventually turning brown (DICA was not as injurious as DDMH). Generally, the higher the concentration of each chemical was, the greater were the stem discoloration and the damage to foliage. A slight chlorosis occurred on all leaves on stems held in DICA or DDMH solutions. Levels of 200 to 300 mg DICA and DDMH/l with and without sucrose were effective in controlling bacteria in water.

Gladiolus.—Flowers held in water did not maintain fresh weight and quality as well as those held in the DICA or DDMH with or without sucrose (table 11). Spikes held in DICA or DDMH with sucrose weighed more and had more open florets and fewer wilted florets after 7 days than did those held in DICA or DDMH without sucrose. In DICA or DDMH alone, flowers were not injured, but in DICA or DDMH with sucrose they developed chlorotic leaves.

Chrysanthemums.—Flowers held in 150 mg of DICA or DDMH developed chlorotic foliage (table 12), and the longer they were held in DICA and DDMH, the greater was the incidence of chlorosis. Leaves on stems held in DICA and DDMH with sucrose were not as chlorotic as leaves on stems held in DICA or DDMH alone. In water, leaves were not chlorotic after 11 days, but in sucrose they were slightly chlorotic and wilted. Flowers held in DICA or DDMH with or without sucrose did not have wilted leaves. DICA or DDMH with or without sucrose effectively reduced or eliminated the bacteria in water.

SECTION 6.—DISCUSSION

As a group, the chlorinating chemicals effectively reduced the bacterial population in cut-flower water. Although effective bactericides, many of the chlorinating chemicals were phytotoxic to chrysanthemums. In the initial screening, both DICA and DDMH at 50 mg/l effectively controlled bacteria and did not injure chrysanthemum leaves. The advantages of these compounds are noteworthy: chemical stability, slow release of chlorine, and control of bacteria at a low solution pH. They also have a relatively long residual effect as bactericides, do not precipitate, and are relatively nontoxic to humans (*14*). Of the two, DICA is more water soluble than DDMH.

TABLE 10.—*Influence of sucrose, and three rates of DICA and DDMH with and without sucrose on bacterial growth, floret display, weight change, and stem discoloration of* 'Oklahoma' *snapdragons*

Chemical concentration/l	Bacterial growth[1]	Floret display[2]			Percent weight change after—		Stem discoloration[3] (cm)
		Initial	5 days	7 days	3 days	5 days	
Water (control)	+ + +	6.2	2.5	([3])	−10	−32	0.3
20 g sucrose	+ + +	7.0	6.0	([3])	−10	−34	.4
100 mg DICA	−	5.7	10.7	4.5	+18	+17	5.4
200 mg DICA	−	6.8	9.5	4.0	+11	+10	7.2
300 mg DICA	−	7.0	9.4	3.6	+13	+8	8.5
100 mg DICA+20 g sucrose	+ +	6.2	11.5	8.0	+9	+9	1.4
200 mg DICA+20 g sucrose	−	7.2	12.9	9.0	+11	+8	3.1
300 mg DICA+20 g sucrose	−	7.8	10.6	11.5	+6	−3	5.2
100 mg DDMH	+	6.8	10.6	3.4	+14	+6	4.9
200 mg DDMH	−	6.2	6.8	1.2	+11	−1	9.1
300 mg DDMH	−	7.0	6.7	1.2	+5	−9	16.1
100 mg DDMH+20 g sucrose	−	6.3	14.8	14.7	+20	+21	8.2
200 mg DDMH+20 g sucrose	−	7.5	17.2	14.6	+15	+17	16.0
300 mg DDMH+20 g sucrose	−	7.2	12.7	11.4	[illegible]	[illegible]	19.1

[1] + = possibility of growth; + + = clear indication of grow
[2] Floret display or viable florets per spike = total flore
[3] Stem discoloration measured after 6 days, then spik

TABLE 11.—*Influence of three rates of DICA and DDMH with and without sucrose on weight change and floret display of* 'White Friendship' *gladiolus spikes*

Chemical concentration/l	Percent weight change after—			Floret display[1]	
	3 days	4 days	7 days	4 days	7 days
Water (control)	+13	−1	−22	3.5	2.2
100 mg DICA	+17	+28	+16	4.4	3.3
200 mg DICA	+20	+31	+21	4.5	3.3
300 mg DICA	+19	+28	+19	3.9	3.3
100 mg DICA + 40 g sucrose	+25	+34	+38	4.5	5.3
200 mg DICA + 40 g sucrose	+25	+43	+53	5.0	6.3
300 mg DICA + 40 g sucrose	+26	+38	+47	4.6	5.5
100 mg DDMH	+15	+33	+20	4.4	3.0
200 mg DDMH	+21	+25	+10	3.4	3.0
300 mg DDMH	+19	+27	+8	3.3	3.5
100 mg DDMH + 40 g sucrose	+22	+37	+40	5.3	6.4
200 mg DDMH + 40 g sucrose	+21	+40	+49	4.3	5.8
200 mg DDMH + 40 g sucrose	+26	+38	+36	5.1	6.0

[1] Floret display or viable florets per spike = total florets open per spike minus number of wilted florets per spike.

Silver (as $AgNO_3$) effectively reduced bacteria in cut-flower water but precipitated, forming a black coating on flower stems. Ag-protein also maintained control of bacteria, but preliminary tests indicated that 20 mg of Ag, supplied as Ag-protein, was phytotoxic to snapdragon leaves. Although the solutions were free of bacteria, flowers wilted prematurely. Ag-protein is a finely divided colloid rather than a solute (*14*), and its physical configuration may have caused the premature wilting. However, the compound should be further investigated on other cut flowers.

TABLE 12.—*Influence of sucrose, and DICA and DDMH with and without sucrose on bacterial populations and incidence of foliar chlorosis in* 'Blue Marble' *chrysanthemums*

Chemical concentration/l	Bacteria/ml at 4 days	Foliar chlorosis after[1]— 5 days	8 days	11 days
Water (control)	2.2×10^5	1.0	1.0	1.0
20 g sucrose	5.3×10^5	1.0	1.2	2.0
150 mg DICA	0	1.5	2.7	3.7
150 mg DICA + 20 g sucrose	([2])	1.2	1.5	3.0
150 mg DDMH	([2])	2.0	3.7	5.0
150 mg DDMH + 20 g sucrose	0	1.0	1.5	2.2

[1] 1 = no chlorosis; 2 = incipient chlorosis; 3 = moderate chlorosis; 4 = moderate chlorosis with necrotic leaf margins; 5 = necrotic leaves.

[2] Lowest dilution was 10^{-2} and only 1 colony found on 1 of 4 plates.

Some quaternary ammonium surface-active disinfectant mixtures (quats) were evaluated on a limited test basis. All mixtures used above 200 mg/l were phytotoxic to chrysanthemums. Bacteria in water were not counted. Quats were not included in preliminary screening because these agents are used in agar medium for the isolation of *Pseudomonas aeruginosa* (*20*). It has been shown that chrysanthemum and other cut flowers can be colonized with *Pseudomonas aeruginosa* (*19, 20*). Various mixtures of quats may control cut-flower bacteria, but these compounds will have to be evaluated in future tests.

Many compounds tested controlled cut-flower bacteria but injured chrysanthemum leaves. Since chlorosis depends on chemical absorption and translocation, it is important that the lowest effective concentration of bactericide be used. In this way the likelihood of chlorosis and phytotoxicity is reduced.

DICA and DDMH effectively maintained low counts of bacteria in water holding chrysanthemums, roses, asters, carnations, and gladiolus (tables 2–5) but not gypsophila (table 6). Although DICA and DDMH reduced the bacteria to low levels, this reduction did not necessarily have a favorable effect on cut-flower quality and longevity. Roses held in DICA and DDMH with sucrose maintained weight and turgor better

than did flowers held in water (table 2), but asters held in DICA or DDMH without sucrose wilted, and their quality was similar to that of flowers held in water. The most dramatic effect on quality and longevity resulted from the use of DICA and DDMH with sucrose.

Roses, asters, carnations, gladiolus, and gypsophila held in 8-HQC plus sucrose were superior to similar flowers held in water (tables 2–6). However, flowers treated with 8-HQC plus sucrose were neither equivalent in quality to nor as longlasting as flowers held in DICA plus sucrose or DDMH plus sucrose. 8-HQC plus sucrose did not control bacteria as adequately as DICA plus sucrose or DDMH plus sucrose (tables 2–5).

Except for gyposphila, all cut-flower solutions containing DICA or DDMH alone or in combination with sucrose were clear, and there was no visible evidence of bacterial growth or contamination. All water and solutions holding gypsophila were heavily contaminated and had high bacterial populations (table 6). Although the bacterial population in solutions was high, the quality of gypsophila florets was improved in DICA and DDMH plus sucrose or in 8-HQC plus sucrose.

In vitro, DICA and DDMH were effective in eliminating soil-borne bacteria (*9*) and pathogenic bacteria (*19, 20*) normally found in cut-flower water (table 7). Most bacteria were killed after a 5-minute exposure to DICA or DDMH, and a 30-minute exposure was effective in killing cells of all bacterial species tested. The common preservative ingredient 8-HQC was not effective in eliminating any bacteria in these tests (in vivo or in vitro) because the bactericidal properties of 8-hydroxyquinoline and its derivatives depend on the addition of metal ions (*2, 3*). All water used in these tests was de-ionized.

Levels of 300 mg DICA or DDMH/l with sucrose were effective in maintaining good bacterial control. However, high concentrations induced foliar chlorosis and other phytotoxic symptoms in roses, snapdragons, and chrysanthemums (tables 8, 10, and 12). The greater the concentration of the chemical was, the greater was the incidence of chlorosis. Snapdragon stems were bleached in DICA and DDMH solutions, the portion of the stem bleached depending on the concentration of DICA or DDMH. Also, the outer petals of roses were injured. All of the injury that occurred from DICA or DDMH did not become apparent until after flowers in water had deteriorated.

If DICA and DDMH were used with sucrose, the benefits in the quality and the longevity of cut flowers and in the sanitation of water would far outweigh the slight disadvantages of phytotoxicity. These chemicals are not fail-safe and need further testing by laboratory workers, by retail florists, and by flower growers to determine their suitability and full potential. Under commercial conditions, flower containers and water quickly become contaminated. DICA and DDMH have the advantage of maintaining better bacterial control than present-day preservatives. At present, DICA and DDMH are not marketed as bactericides for cut flowers. These compounds are used extensively in many industrial products, bleaches, deodorizers, detergents, dishwashing compounds, and swimming-pool additives (*14*).

Because DICA and DDMH control bacteria, these chemicals may have to be classified as pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act as amended. By definition of the act, the term "pest" includes any "form of terrestrial or aquatic plant or animal life or virus, bacteria, or other micro-organism," and the term "pesticide" includes "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest." Since vase-water bacteria qualify as "pests" and DICA and DDMH qualify as "pesticides," these chemicals will probably have to be registered by the Environmental Protection Agency before being made available for commercial use as controllers of cut-flower bacteria.

SECTION 7.—LITERATURE CITED

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